Cytotoxicity Studies to Characterize Self-Assembling Amphiphiles for Targeted Cancer Drug Delivery

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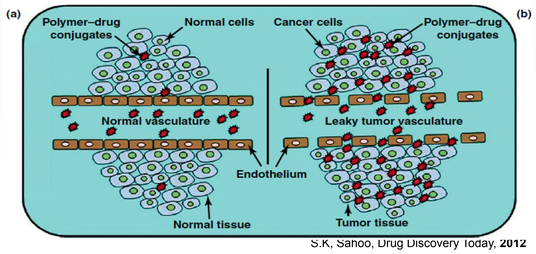
Background

Advantages of nanodrugs in anticancer research

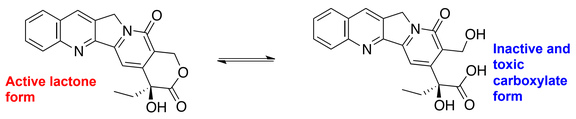
- Improves water solubility and drug stability
- Prolongs circulation time
- Increased target specificity
- Facilitates passive accumulation in tumors via enhanced permeation and retention effect



S.K, Sahoo, Drug Discovery Today, 2012

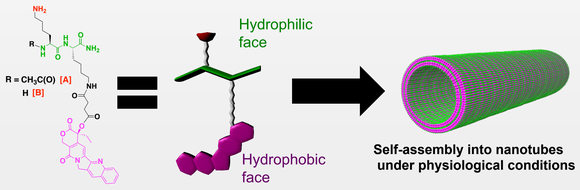
Camptothecin (CPT)

- Possesses potent antitumor properties that derive from its inhibition of topoisomerase I
- Showed remarkable anticancer activity in preliminary clinical trials
- Drawbacks: poor solubility under physiological conditions and unstable E-lactone ring



Self-Assembly of CPT-peptide conjugates

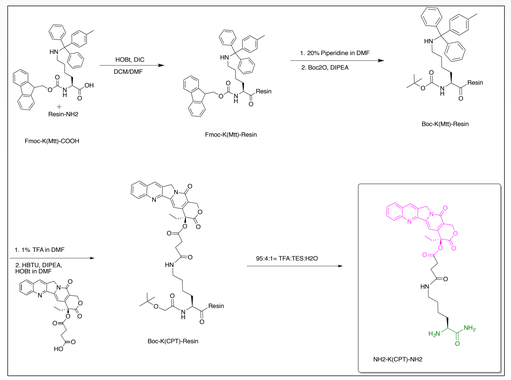
- Peptide conjugation to CPT increases stability of drug
  - Preliminary data testing stability shows hydrolysis occurs at ester bond, not at lactone
- Hydrophilic portion (peptide) and hydrophobic portion (CPT) spontaneously assemble into nanotubes
- Once inside tumor cells, esterases will cleave ester bond → CPT will open to toxic form
- We have developed mono-, di-, and tetra-peptide conjugates



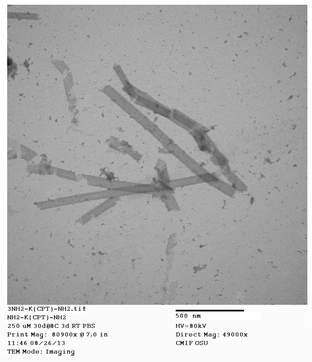
Analysis of Drug

Compound

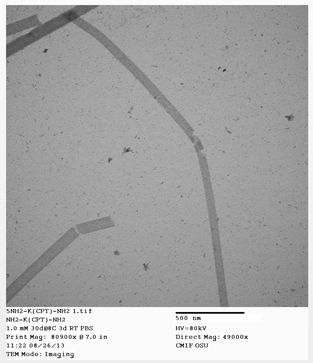
- Compound of interest is NH_2-K(CPT)-NH_2
- Monopeptide derivative is appealing due to simplicity, as opposed to di- and tetra-peptide
- Amide derivative is most favorable due to increased solubility, as opposed to methyl ester and free acid derivatives



Imaging



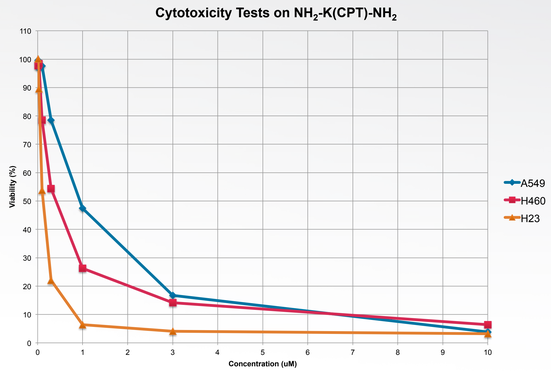
Nanotube formation – 250 μM in Phosphate Buffered Saline (PBS), was diluted from 10 mM stock solution (5.75 μg of drug in 1.0 mL of PBS) that was stored at 4 °C for 1-month, aged for 3-days 23 °C



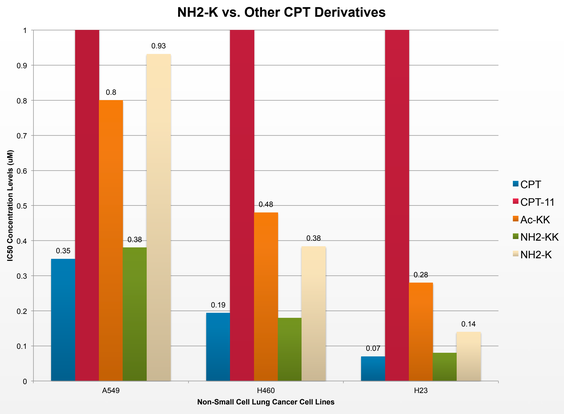
Nanotube formation – 1 mM in PBS, was diluted from 10 mM stock solution (5.75 μg of drug in 1.0 mL of PBS) that was stored at 4 °C for 1-month, aged for 3-days 23 °C

Analysis of Drug

Cytotoxicity Tests



- Cytotoxicity tests were conducted to determine half maximal inhibitory concentration (IC_{50}) levels to test efficacy of drug
- 3 non-small cell lung cancer cell lines (A549, H460, H23) with varying BCRP expression were used
- Drug was administered at varying concentrations and incubated with cells for 96 h
- Concentrations were diluted with PBS from 10 mM stock solution (5.75 μg of drug in 1.0 mL of PBS) that was aged at 23 °C for 3 days, stored at 4 °C in between trials
- Preliminary data

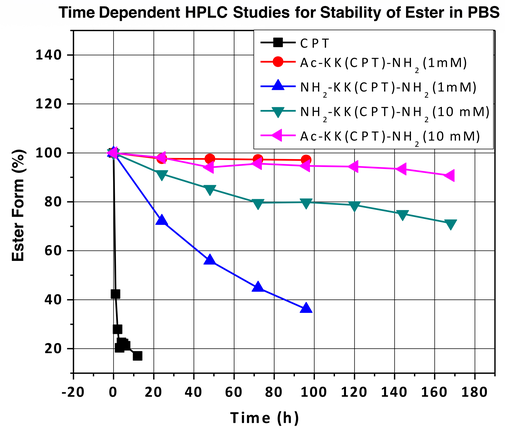


- IC_{50} levels of NH_2-K were compared with CPT, CPT-11 (clinically approved), NH_2-KK, and Ac-KK
- NH_2-K appeared to be comparable to Ac-KK and less effective than NH_2-KK

Conclusions

- NH_2-K(CPT)-NH_2 shows promise based on collected data (TEM and cytotoxicity) so far
- There is formation of nanotubes due to amphiphilicity of molecule
- Cytotoxicity studies
  - Seemed to have similar effectiveness as Ac-KK, but is less effective than NH_2-KK
  - Still more effective than CPT-11 (clinically approved drug)

Future Steps



- Run E-lactone stability tests at pH 7.4 using high performance liquid chromatography
- Run more cytotoxicity trials to confirm preliminary data
- Quantitate accumulation of drug *in vitro* via flow cytometry
- Examine drug flow in cells with confocal images
- Determine biodistribution *in vivo*

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